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ORIGINAL REPORT

Intratumoral Epidermal Growth Factor Receptor Antisense DNA Therapy in Head and Neck Cancer: First Human Application and Potential Antitumor Mechanisms

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Clinical Trials repository link available on JCO.org.

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
Squamous cell carcinoma of the head and neck (SCCHN) is characterized by upregulation of the epidermal growth factor receptor (EGFR). We developed a novel strategy to target EGFR by using a therapeutic gene that consisted of an EGFR antisense (AS) gene sequence under U6 promoter control. A phase I clinical trial was conducted to evaluate the safety and biologic effects of EGFR AS.

Patients and Methods
Patients with advanced SCCHN who were refractory to standard therapies and who had at least one assessable and accessible lesion were enrolled. The EGFR AS dose was escalated in successive cohorts (six dose levels; 60 to 1,920 μg/injection). Patients received four weekly intratumoral EGFR AS injections. Tumor biopsies were performed before and after completion of therapy. Treatment response was assessed by tumor volume measurements (positron emission tomography/computed tomography), and levels of target proteins were assessed by immunohistochemistry.

Results
Seventeen assessable patients were treated. No grades 3 to 4 or dose-limiting toxicities were noted, and a maximum-tolerated dose was not reached. Five patients (29%) achieved a clinical response, which included two complete responses (CRs) and three partial responses (PRs); two additional patients had stable disease (SD) as the best response. Patients with disease control (CR + PR + SD) had tumors with higher EGFR and lower STAT3 expression at baseline compared with patients who had progressive disease ($P = .0312$ and $P = .095$, respectively).

Conclusion
Intratumoral EGFR AS was safe and resulted in antitumor activity in patients with advanced SCCHN. Baseline levels of high EGFR and low STAT3 may be associated with antitumor effects.

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INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN) affects approximately 650,000 patients worldwide, and there are approximately 46,000 new patient cases per year in the United States.[1,2] Approximately two thirds of SCCHN patients present with locoregionally advanced disease (ie, American Joint Committee on Cancer stages III to IV). Standard therapies for SCCHN remain suboptimal and may result in substantial toxicities.[3,4] The development of more precisely targeted therapeutic agents is desirable.

Cumulative evidence suggests that epidermal growth factor receptor (EGFR) overexpression and increased signaling through the receptor complex are critical in the development and progression of some epithelial cancers.[5] EGFR levels correlate with survival independent of other clinical and pathologic parameters, including nodal staging.[6,7] A variety of therapeutic approaches have been developed to block EGFR, including monoclonal antibodies (eg, cetuximab) and tyrosine kinase inhibitors (TKIs; eg, erlotinib). Although cetuximab was recently approved for concurrent use with radiation therapy for SCCHN treatment, cetuximab and EGFR TKIs have relatively low clinical response rates when administered as single agents in recurrent/metastatic SCCHN.[8-11] Additionally, there has been no consistent correlation between EGFR expression and signaling activity or their modulation and the clinical activity of these EGFR-targeted

agents.[12-14] The discordance between preclinical activity of EGFR-targeted agents, their effect(s) on EGFR expression/signaling activity, and their clinical activity suggest that alternative approaches to inhibit EGFR signaling may be more effective.

We developed an EGFR antisense (AS) strategy to target EGFR production directly. Introduction of EGFR AS oligonucleotides into SCCHN cells inhibited proliferation and appeared to be more effective than other anti-EGFR agents.[15] Similarly, antitumor effects were seen when EGFR AS gene therapy (referred to simply as EGFR AS) was safely administered in a nude mouse xenograft model.[16-18] This study was designed to determine the toxicity and safety of intratumoral EGFR AS in patients with SCCHN, and a secondary objective was to examine the effects of EGFR AS treatment on candidate biomarkers in tumor specimens.

PATIENTS AND METHODS

Construction and Production of pNGVL1-U6-EGFRAS

The 39 base-pair EGFR AS sequence spans the translation start site for the *EGFR* gene (5′-CCG GCC GTC CCG GAG GGT CGC ATC GCT GCT CCC CGA AGA-3′).[18] The human U6 promoter and EGFR AS sequence were inserted into a modified pNGVL vector. Plasmid DNA was produced under good manufacturing practice conditions at the Center for Biomedicine and Genetics at the City of Hope (Duarte, CA) to City of Hope's Master File BB-MF-9778.

Clinical Trial

We implemented a rapid dose escalation (100% increment increased between tiers) at 1 μg/μL of DNA: 60 μg; 120 μg; 240 μg; 480 μg; 960 μg; and 1,920 μg. Three patients were enrolled per dose tier and were monitored for at least 2 weeks before patients were enrolled at the next level. In this classic 3 + 3 design, criteria were established to enroll three additional patients at a given dose level if one of the first three patients experienced a dose-limiting toxicity (DLT). DLTs were defined as grades 3 to 4 toxicities, as enumerated by the National Cancer Institute Common Toxicity Criteria and included hemorrhage at the injection site and hemodynamic compromise. Patients were enrolled serially onto the study without modification of gene dose relative to tumor size.

All patients who were enrolled had advanced SCCHN disease refractory to standard therapies, including surgery, radiation therapy, and/or chemotherapy (Table 1). Each patient had been treated with at least two treatment modalities sequentially or concurrently and had an Eastern Cooperative Oncology Group performance status of 0 to 2. Written informed consent was obtained from each participant.

Patients underwent physical examination and laboratory evaluation. Baseline disease extent was assessed within 6 weeks of starting treatment by positron emission tomography (PET)–computed tomography (CT). To assess the efficacy of EGFR AS treatment by dose, the most accessible single lesion was selected for biopsy and injection of EGFR AS in the outpatient setting. EGFR AS was injected into the lesion under direct visualization by dividing the target into quadrants and injecting an equal volume into each quadrant of the tumor weekly for 4 weeks. No supportive measures (eg, anti-inflammatory agents) were given with the injection. Within 2 weeks after the final injection, another biopsy was performed at the monitored site. Biomarkers were assessed in the specimens by immunohistochemistry. Patients received a second PET-CT scan approximately 6 weeks after completion of therapy. No other concomitant anticancer therapy was given. Response to treatment was evaluated according to modified RECIST (Response Evaluation Criteria in Solid Tumors) and focused on a single index lesion.[20] Disease control was defined as either complete response (CR), partial response (PR), or stable disease (SD).

This phase I clinical trial was approved by the University of Pittsburgh institutional review board. Treatment and tumor biopsies were performed in the University of Pittsburgh General Clinical Research Center, Pittsburgh, PA.

Table 1. SCCHN Patients Treated With EGFR AS Gene Therapy

Patient	Age (years)	Sex	TNM Stage	SURG	Prior Treatment XRT	CHEMO	EGFR AS Dose (μg)	Lesion Injected	Maximum Diameter (cm)	Clinical Response	Status	TTP (mos)	F/U (months)
1	47	M	T3N1M0	Y	Y	Y	60	R neck	7.5	PD	Deceased	1.4	3.3
2	75	M	T2N2M0	Y	Y	Y	60	R neck	2.8	SD	Deceased	6.5	7.7
3	60	M	T2N0M0	Y	Y	N	60	R alveolus	5.0	PD	Deceased	1.4	1.4
4	54	M	T2N0M0	Y	Y	Y	120	R tonsil		*			
5	67	M	T2N0M0	Y	Y	N	120	R RMT	1.4	CR	Deceased	26.2	29.9
6	57	M	T4N2M0	N	Y	Y	120	L RMT	2.6	PR	Deceased	6.5	7.9
7	45	M	T2N1M0	Y	Y	Y	120	R FOM	7.3	PD	Deceased	1.4	4.0
8	46	M	T2N2M0	Y	Y	Y	240	L tongue		*			
9	55	M	T2N2M0	Y	Y	Y	240	L tonsil	2.4	PR	Deceased	—	5.3
10	45	M	T2N0M0	Y	Y	Y	240	L FOM	5.6	PD	Deceased	1.4	10.4
11	50	M	T2N3M0	N	Y	Y	240	R neck	4.0	PD	Deceased	1.4	2.5
12	53	M	T3N1M0	N	Y	Y	480	L tongue	1.3	PD	Deceased	1.4	3.4
13	74	M	T2N1M0	Y	Y	Y	480	R neck	2.2	PD	Deceased	1.4	2.6
14	45	F	T2N0M0	N	Y	Y	480	R neck		*			
15	53	F	T4N1M0	Y	Y	Y	480	R tongue	1.0	CR	Deceased	NED	8.3
16	71	M	T2N0M0	Y	Y	Y	960	L neck	4.8	SD	Deceased	3.7	5.4
17	76	M	T2N1M0	N	Y	Y	960	L palate	2.7	PR	Deceased	—	9.9
18	47	M	T4N2M0	N	Y	Y	960	R FOM	2.7	PD	Deceased	1.4	1.7
19	66	F	T2N0M0	Y	Y	N	1920	R FOM	6.3	PD	Alive	1.4	21.0
20	59	M	T2N1M0	N	Y	Y	1920	L postauricular	4.0	PD	Deceased	1.4	25.6

NOTE. TNM stage was determined by 2002 American Joint Committee on Cancer guidelines.[19]

Abbreviations: SCCHN, squamous cell carcinoma of the head and neck; EGFR, epidermal growth factor receptor; AS, antisense; SURG, surgery; XRT, radiation therapy; CHEMO, chemotherapy; TTP, time to progression; F/U, follow-up; PD, progressive disease; SD, stable disease; RMT, retromolar trigone; CR, complete response; PR, partial response; FOM, floor-of-mouth; NED, no evidence of disease.

*Nonassessable participant.

Statistical Methods

Patients were classified into response categories: disease control (CR + PR + SD) or clinical response (CR + PR). Differences between two patient groups were compared with the Wilcoxon Test. Within-patient differences were tested with the signed rank test. Immunohistochemical scores were tested for a trend across the four clinical response categories by assigning arbitrary scores to the four response categories to reflect ordering from best to worst response. The log odds of a clinical response as a function of dose and initial tumor size were described by fitting logistic regression models. PET measurements (standardized uptake value [SUV]) were tested for association with initial tumor volume by ordinary least squares regression. All regression models were assessed for adequacy of fit.

RESULTS

Patient Cohort and Adverse Events

Twenty patients with advanced SCCHN disease refractory to standard therapies were enrolled on this study (Table 1). Seven patients had distant metastases that were not accessible for intralesional injection. If multiple lesions were noted at presentation, a single, accessible lesion was selected for EGFR AS intratumoral injection. One patient died as a result of disease before assessment of tumor response, and two patients died as a result of disease before completion of all four injections. Two deaths were caused by tumor that encased the carotid artery that was distant from the EGFR AS injection site. Seventeen patients completed the entire treatment course and were assessable for toxicity (median age, 57 years; 15 men, 2 women). No grades 3 to 4 toxicities or DLTs were noted, and the maximum-tolerated dose was not reached. Although the original goal was to identify DLTs and the maximum-tolerated dose, patient enrollment and treatment continued until no more clinical-grade EGFR AS plasmid was available. Three grade 1 treatment-related toxicities were reported, including injection site pain/swelling (n = 2) and localized edema (n = 1) in only two patients (patient 1 at 60 μg and patient 20 at 1,920 μg).

Overall EGFR AS Antitumor Efficacy

Tumors were injected with EGFR AS sequence under the control of a U6 promoter (Fig 1A) at the anatomic sites listed in Table 1. In the assessable cohort, nine patients were treated at mucosal sites of recurrent disease, and five patients had a cervical lymph node metastasis injected with EGFR AS. Three patients were treated at the site of a new primary tumor that was encompassed within a previous treatment field, precluding additional conventional therapy. Median tumor size and standard deviation was 2.8 $\pm$ 2.0 cm (range, 1.0 to 7.5 cm). We were able to detect the EGFR AS gene by polymerase chain reaction in all participants, as demonstrated in the representative patient panel (Fig 1B).

We evaluated disease response to treatment at the monitored site by employing modified RECIST to assess the target index lesion at the site of injection.[20] Objective clinical responses were achieved in five patients (29%; 95% CI, 10% to 56%), which included two CRs and three PRs (Fig 1C). Two patients had SD as the best response, which provided a disease-control rate of 41% (95% CI, 18% to 65%). Median duration of response or time to progression (TTP) at the target lesion for the entire cohort was 1.4 months. In the disease-control group, TTP was increased at 6.5 months (95% CI, 3.0 to 23.1 months). Three patients survived for extended periods of time after EGFR AS. Patients 19 and 20 survived for 21.0 and 25.6 months, respectively, although they had progressive disease (PD) at the selected injection site. Both declined additional treatment. Patient 5 did not have disease progression for 26.2 months and then was treated with cetuximab until he died at 29.9 months.

To identify potential clinical parameters related to clinical response, we created logistic regression models that related clinical response to EGFR AS dose and to maximum tumor dose. Clinical response appeared to be associated with the reciprocal of tumor diameter and not to EGFR AS gene dose (Fig 1D). The model demonstrated that smaller tumors were more likely to respond and that the probability of response declines sharply with increasing maximum tumor diameter ($P = .0592$).

The Kaplan-Meier estimate of median survival was 5.4 months (95% CI, 2.6 to 9.9 months). In the disease-control group, median survival was 7.9 months (range, 5.3 to 29.9 months) compared with 3.4 months for patients with PD.

Correlation of Biomarker Expression With EGFR AS Treatment

Given the relatively high overall response rate, we explored potential antitumor mechanisms for EGFR AS. Original H&E stained slides were reviewed to verify disease response. In two patients with CR, the pretreatment specimen demonstrated SCCHN, but only dysplasia, in the post-treatment specimens (Fig 2A). In contrast, patients with PD had SCCHN in both the pretreatment and post-treatment specimens (data not shown). Thus, in patients with a CR to EGFR AS, the lesion at the injection site demonstrated pathologic regression.

Although tissue specimens were acquired from all patients pre- and post-treatment, several specimens did not contain a sufficient number of viable tumor cells to analyze biomarker expression by immunohistochemistry. From the 17 assessable patients, 11 had sufficient baseline tissue for EGFR staining, and eight had paired specimens. Paraffin-embedded specimens were utilized when available for biomarker assessment. EGFR expression was quite uniform and intense in the pretreatment specimen for patients with a CR compared with patients with PD (Fig 2C). We identified an association between baseline EGFR expression in the pretreatment specimens and disease control ($P = .0312$; Fig 2D). As response to EGFR AS represents a continuum from CR to PD, we assessed the association between EGFR expression and EGFR AS response. A two-tailed exact Jonckheere's test for trend supports this association between EGFR expression and EGFR AS response ($P = .0271$; Fig 2E).

In the eight paired specimens, we noted a significant overall decrease in EGFR expression by immunostaining when comparing pre- and post-treatment specimens ($P = .0172$; Fig 2B). A comparison of EGFR immunohistochemical staining before and after EGFR AS suggests a trend for a larger decrease in EGFR levels in patients who experienced disease control compared with patients who had PD ($P = .1771$; Fig 2F). An alternative grouping that compared clinical response (CR + PR) to SD + PD supports this potential association ($P = .0583$; Appendix Fig A1, online only). Thus, the degree of downregulation in EGFR expression may be associated with clinical response, although we caution against overinterpretation, as these are exploratory findings in a small, phase I, clinical trial patient cohort.

We also performed immunohistochemistry studies for STAT3, pSTAT3, pSTAT5, and pAkt, but we did not find any significant changes in the expression of these proteins after EGFR AS treatment in

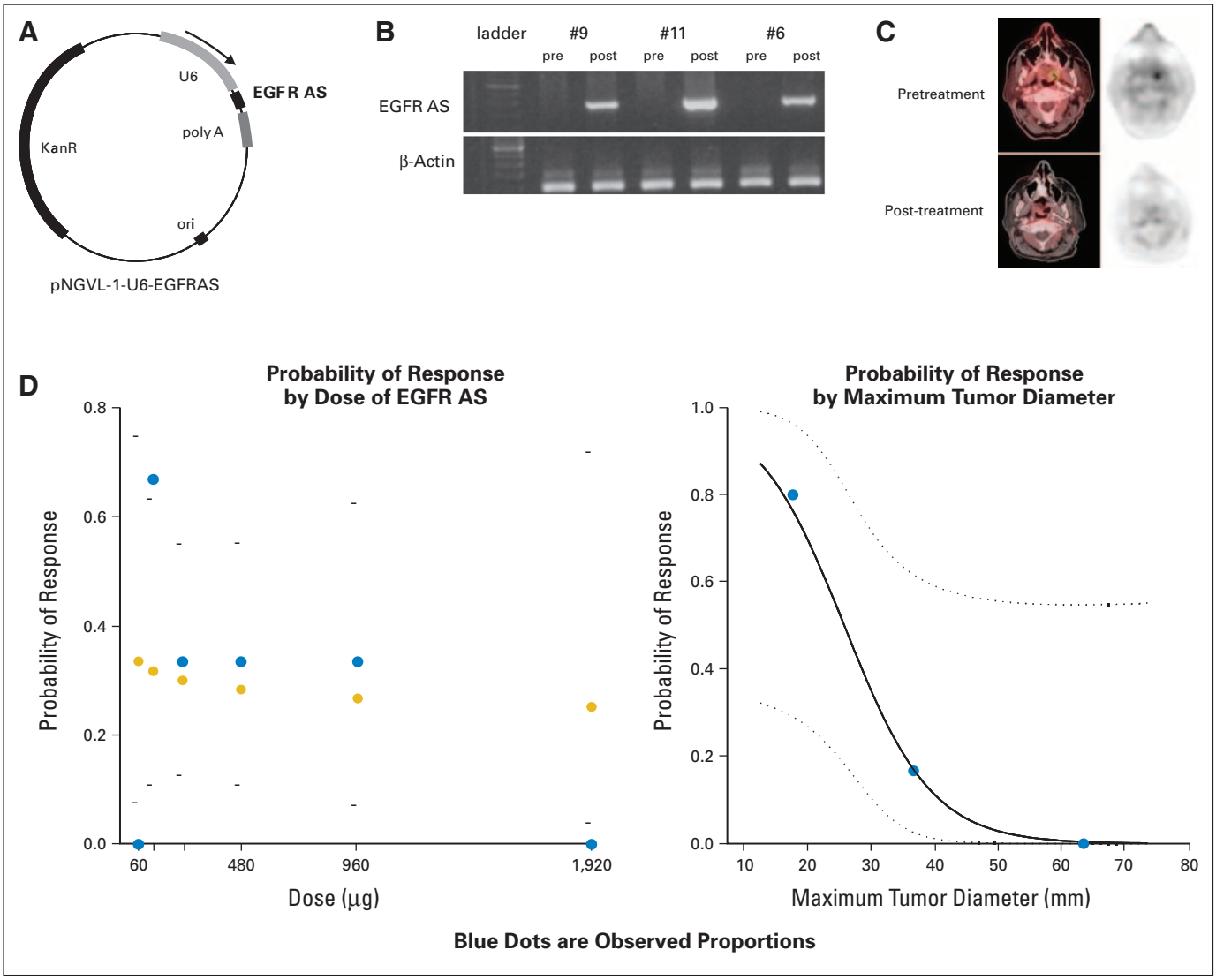

Fig 1. Treatment with the epidermal growth factor receptor antisense (EGFR AS) gene. (A) pNGVL1-U6-EGFRAS contains an EGFR AS gene under the control of the U6 promoter into the pNGVL1 vector, which contains a kanamycin-resistance (KanR) marker. Clinical-grade plasmid DNA was produced by the Center for Biomedicine and Genetics at the City of Hope (Duarte, CA). (B) EGFR AS was measured by polymerase chain reaction in DNA extracted from representative pre- and post-treatment patient biopsy specimens. Patients 6 and 9 had a partial response to treatment, whereas patient 11 had no response to EGFR AS. β-actin served as a loading control. (C) Axial positron emission tomography–computed tomography (PET-CT) images of patient 17 demonstrate partial response to EGFR AS. Patient 17 received an EGFR AS dose of 960 μg per injection, and the PET-CT scan images were performed within 6 weeks of treatment initiation and immediately after treatment completion. The tumor present on the hard palate (white arrow top left panel) regressed significantly after EGFR AS, which resulted in an open defect in that area (white arrow lower left panel). A concomitant decrease in PET activity was seen in the treatment area (right panels). (D) The relationship between response to EGFR AS therapy and EGFR AS dose or maximum tumor diameter was examined. Blue circles indicate the observed proportions of responders at each gene dose level. Yellow circles (left) and solid line (right) are the response probabilities from logistic regression models. Dotted lines represent 95% confidence bands for the predicted values. The wide bands are consistent with a model with only 17 observations.

patients, regardless of clinical response (data not shown). Examination of baseline STAT3 expression demonstrated a correlation with disease control. Patients who experienced CR had low STAT3 expression pretreatment (Fig 3A; data not shown). In contrast, patients who experienced PD demonstrated both uniform and intense STAT3 antibody reactivity in the pretreatment specimen. The difference in STAT3 expression levels in the disease-control group compared with patients who experienced PD demonstrated a trend toward significance ($P = .095$; Fig 3B). Additionally, a two-tailed exact Jonckheere's test for trend demonstrated a significant association between decreased pretreatment STAT3 expression and EGFR AS response ($P = .0381$; Fig 3C). There were too few samples available from patients in this trial to assess the association between high EGFR expression and low STAT3 expression.

Assessment of EGFR AS Treatment Response by [^{18}F]Fluoro-2-Deoxyglucose PET Scan

We measured the change in maximum SUV (SUV_{max}) and mean SUV (SUV_{mean}) at the site of EGFR AS injection in our patients (Appendix Table A1, online only). In patient 16 (who experienced SD), there was a striking decrease in SUV_{max} of the treated left neck lesion from 14.6 to 4.5 (Appendix Fig A2A, online only). This patient

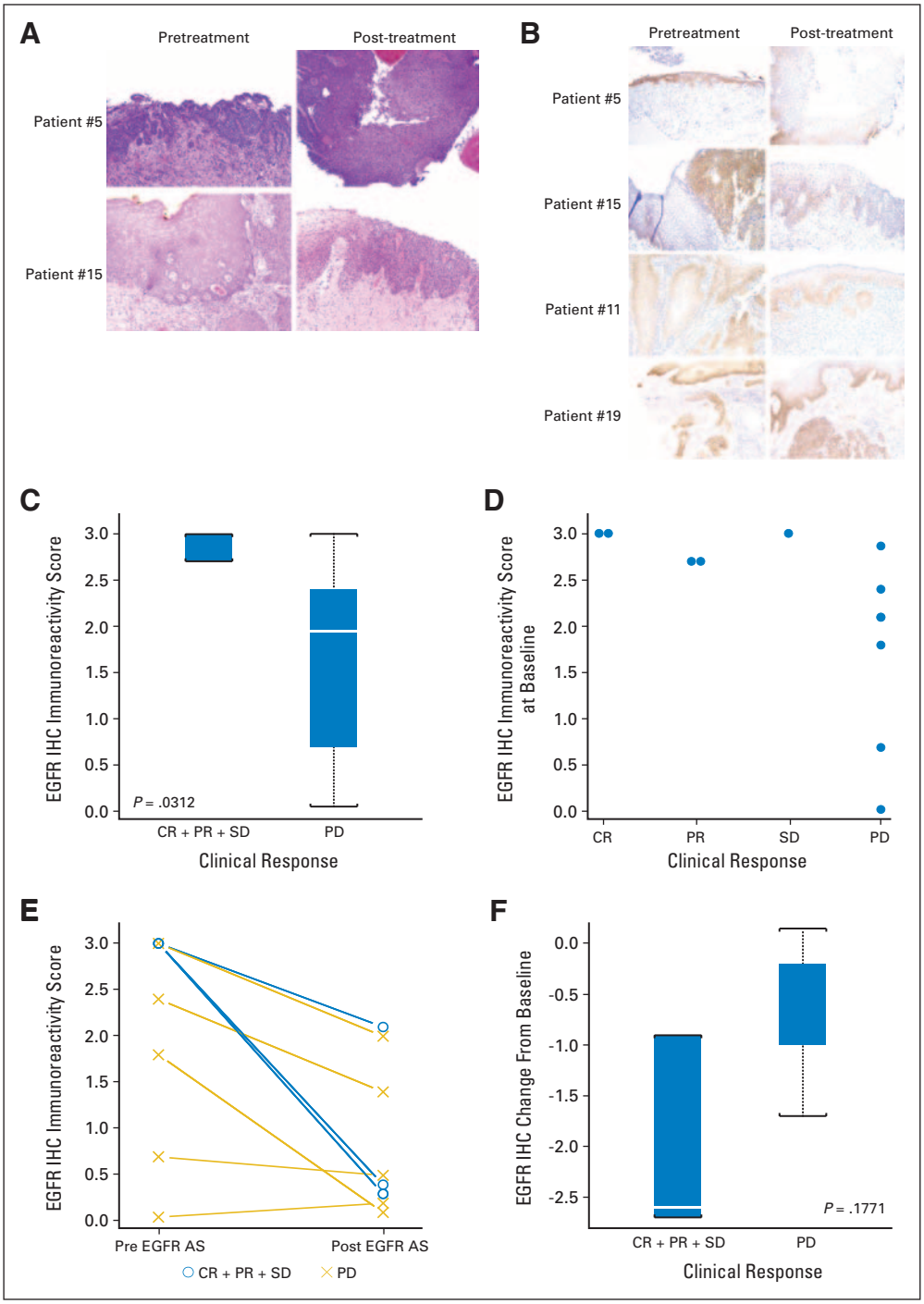

Fig 2. Pre- and post-treatment patient specimens demonstrate tumor response and modulation of epidermal growth factor receptor (EGFR) expression. (A) Patient specimens were prepared with hematoxylin and eosin staining and were examined for the presence of tumor before and after EGFR antisense (AS; ×10 magnification). Patient 5 pretreatment specimen demonstrates superficially invasive squamous cell carcinoma; post-treatment biopsy demonstrates only mild-moderate dysplasia. Patient 15 has invasive squamous cell carcinoma with infiltrative fronds that extend into stroma present in the pretreatment specimen. Severe dysplasia is seen with no residual invasive disease after EGFR AS. (B) The expression of EGFR in the patient tissue specimens with complete response (CR; top) and progressive disease (PD; bottom) were examined by immunohistochemistry. Patient 5 pretreatment carcinoma shows strong EGFR antibody reactivity. The post-treatment specimen demonstrates patchy EGFR antibody reactivity that is diminished in the mucosa. Patient 15 pretreatment carcinoma is strongly reactive to EGFR antibody compared with the adjacent non-neoplastic mucosa. The post-treatment dysplastic mucosa shows diminished EGFR antibody reactivity. In both patients 11 and 19, the pretreatment and post-treatment carcinomas show EGFR antibody reactivity without significant change after EGFR AS. All photomicrographs were taken at ×100 original magnification. (C) The box-and-whisker plot illustrates the differences in pretreatment EGFR immunohistochemical staining scores between patients with disease control (CR, partial response [PR], or stable disease [SD]) and those with PD ($P = .0312$). (D) A scatter plot demonstrates the relationship between pretreatment EGFR immunohistochemical staining scores and the spectrum of clinical responses from CR to PD ($P = .0271$). (E) Expression of EGFR in paired patient specimens before and after EGFR AS. EGFR expression was decreased in all except one of the paired specimens ($P = .0172$). (F) The box-and-whisker plot demonstrates the change in EGFR immunohistochemical staining scores between pre- and post-treatment specimens when comparing patients with disease control (CR, PR, SD) and those with PD ($P = .1771$).

had no significant disease progression for 3.7 months and survived for 5.4 months after EGFR AS. SUV_{max} decreased in five of seven patients who experienced PD (median ΔSUV_{max}, 0.7), despite increasing tumor volume. Although a number of patients who experienced PD survived with disease for an extended period of time, we were not able to identify a significant association between ΔSUV_{max} and survival in a Cox proportional hazards model ($P = .23$). In the development of the logistic regression model, we did note a correlation between pretreatment tumor size and baseline SUV_{max} (Appendix Fig A2B). Comparison of the disease-control and PD groups demonstrated a likely association between a low pretreatment SUV_{max} and response to EGFR AS ($P = .0513$; Appendix Fig A2C). This finding confirms the association between clinical response and tumor diameter (Fig 1D).

DISCUSSION

Anti-EGFR monoclonal antibodies (eg, cetuximab) and TKIs (eg, erlotinib) are currently the most developed EGFR-targeted therapies.[11] A phase III, randomized trial of radiation therapy with or

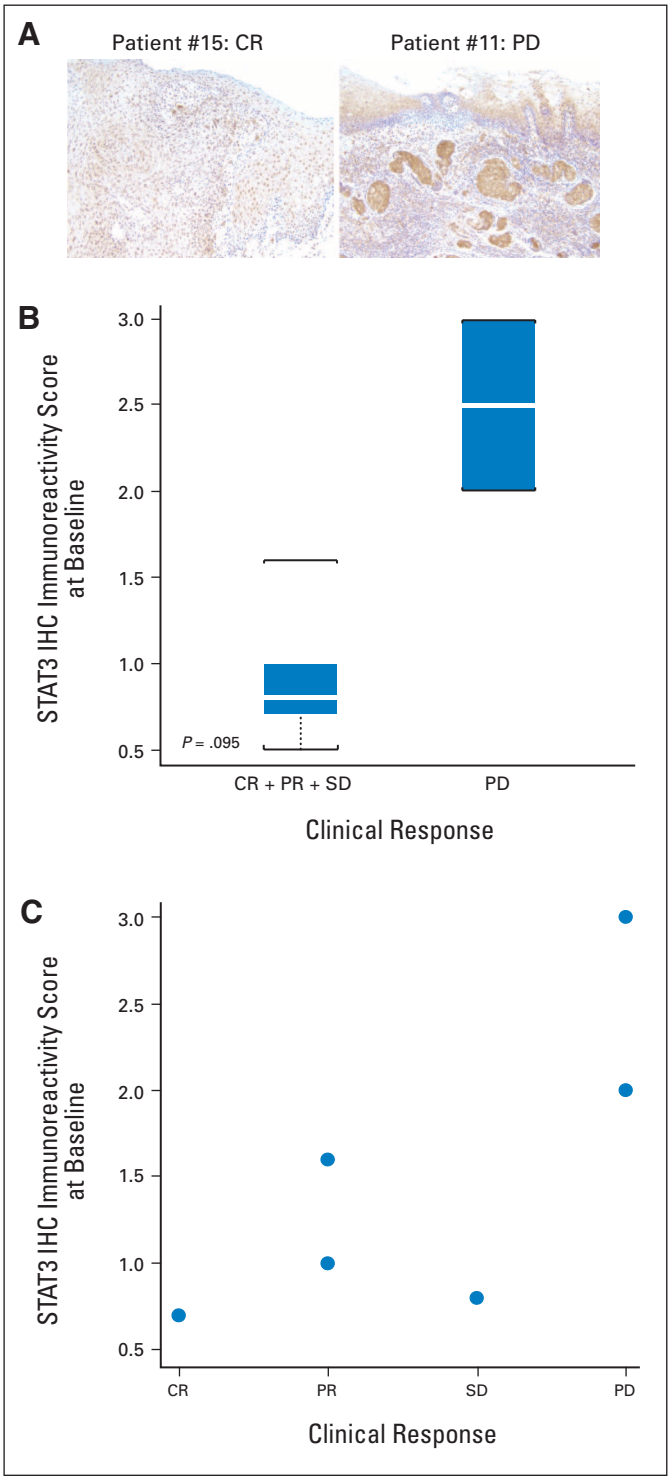

Fig 3. Pretreatment patient specimens demonstrate differences in baseline STAT3 expression between patients with disease control compared with progressive disease (PD). (A) The expression of STAT3 in pretreatment patient tissue specimens was examined by immunohistochemistry. The pretreatment lesion in patient 11 with PD demonstrated stronger STAT3 antibody reactivity than the lesion in patient 15 with complete response (CR). All photomicrographs were taken at ×100 original magnification. (B) The box-and-whisker plot illustrates the differences in pretreatment STAT3 immunohistochemical staining scores between patients with disease control (CR, partial response [PR], stable disease [SD]) and those with PD ($P = .095$). (C) The scatter plot demonstrates the relationship between pretreatment STAT3 immunohistochemical staining scores and the spectrum of clinical responses from CR to PD ($P = .0381$).

without cetuximab demonstrated increased survival in patients with stages III to IV SCCHN without worsening in-field radiation toxicities, which led to US Food and Drug Administration approval of this agent in SCCHN.[21] Despite the clear molecular rationale for their development, use of these agents as monotherapy or in conjunction with conventional cytotoxic chemotherapy agents in a number of phase I/II trials for SCCHN and NSCLC has consistently demonstrated only a 5% to 15% clinical response rate.[8-10,12,22] This response rate is consistent with the historical activity of chemotherapy agents as monotherapy in phase I clinical trials.[23]

Intratumoral injection of EGFR AS resulted in a 29% clinical response rate at the site of injection (2 CRs and 3 PRs among 17 assessable patients; 95% CI, 10% to 56%), and two patients had SD, which provided a disease-control rate of 41% (95% CI, 18% to 56%). Increased clinical response rates with EGFR AS may be due to targeting transcriptional regulation and downregulating protein levels, which may be more effective than blockade of EGFR ligand-binding or tyrosine phosphorylation. However, we caution against overinterpretation of these results, as the primary objective of our phase I clinical trial was assessment of feasibility and safety for EGFR AS treatment. Additionally, any direct comparison of systematically delivered agents with intratumoral EGFR AS requires additional investigation.

Given the relatively high overall response rate to EGFR AS, we explored potential molecular mechanisms for EGFR AS in patients with SCCHN, despite the relatively small size of this phase I clinical trial patient cohort. Assessment of the pretreatment tumor specimens identified higher EGFR expression ($P = .0312$) and lower baseline STAT3 levels ($P = .095$) in the disease-control group. The oncogenic function of STAT3 in epithelial cancers and its role in EGFR signaling[24,25] provide a biologic explanation for these findings. We validated this mechanism in vitro, where the antiproliferative effects of EGFR downregulation were enhanced when expression of STAT3 was concomitantly decreased (Appendix Fig A3, online only). This finding suggests that SCCHN in some patients may demonstrate a specific dependence on certain oncogenic pathways (eg, EGFR). Increased expression of EGFR may be a marker for such dependence, and we propose that downregulating EGFR may be most effective in those tumors with high EGFR levels in which these cells are unable to compensate through other oncogenic signaling pathways, such as STAT3.

EGFR AS decreased EGFR expression in the treated tumor across all of the assessable specimen pairs except for one. In patients with disease control, there was a relatively larger decrease in EGFR expression after EGFR AS treatment, which suggests that the degree of target downmodulation may predict clinical response. In contrast, a relationship between EGFR levels and related signal transduction molecules with response to EGFR TKIs or anti-EGFR monoclonal antibodies has not been well established.[12-14] This may be a result of the targeting of transcriptional regulation by EGFR AS rather than the overexpressed EGFR protein, which has not been reported as a mechanism of action for EGFR TKIs or anti-EGFR monoclonal antibodies.

A limitation of this study was the challenge to procure high-quality tumor specimens for biologic assessment. Despite this limitation, our finding that EGFR (both baseline and degree of downregulation) and STAT3 expression levels were associated with a clinical response to EGFR AS suggests that assessment of target expression in an individual tumor may identify those patients who are most

likely to respond to a specific targeted therapy. Despite the relatively small number of patients in this phase I study, our experience and other studies[14] emphasize the importance of acquiring both pretreatment and post-treatment specimens in clinical trials that use targeted therapies.

Intratumoral delivery of a therapeutic agent has been attempted in a variety of diseases, including cancer.[26] A number of these agents consisted of gene therapy approaches, including immunotherapy, oncolytic viral therapy, and gene transfer.[27,28] In this study, we did not employ a viral vector but instead relied on the U6 promoter for gene delivery, which, to our knowledge, makes this the first human trial to administer U6-driven gene therapy. Although SCCHN lesions tend to be more accessible for intratumoral injection, such an approach is limited by the ability to ensure uniform delivery to the entire lesion and to identify and treat multiple lesions. The development of a modified nucleotide/peptide hybrid that would be stable for systemic administration is currently in process.

Although the assessment of clinical response in cancer patients to new therapeutic agents has traditionally relied on target lesion size changes, [^{18}F]fluoro-2-deoxyglucose (FDG)-PET imaging may also provide useful pretreatment information and/or predict disease response. Although the SUV_{max} change did not correlate with disease response/survival, SUV_{max} of the treatment site correlated with overall tumor volume. The clinical parameter most clearly associated with clinical response was the baseline volume of the injected SCCHN lesion ($P = .0592$). Similarly, a lower pretreatment SUV_{max} was predictive of a disease-control response ($P = .0513$). The role of PET-CT scans continues to evolve in the assessment of tumor progression and response to therapy.[29]

This study demonstrates the safety and lack of toxicity of direct injection of an EGFR AS gene into SCCHN tumors. EGFR AS was not associated with significant toxicities, and no DLT was reached, which is consistent with previous studies that demonstrated negligible toxicity from intratumoral DNA administration.[30,31] In this small cohort, EGFR AS demonstrated promising antitumor activity, in which EGFR and STAT3 expression levels were associated with response to this EGFR-targeted therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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